

LAW OFFICES
SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC
 2100 PENNSYLVANIA AVENUE, N.W.
 WASHINGTON, DC 20037-3213
 TELEPHONE (202) 293-7060
 FACSIMILE (202) 293-7860
 www.sughrue.com

January 13, 2000

BOX PCT

Assistant Commissioner for Patents
 Washington, D.C. 20231

PCT/JP98/02927
 -filed June 30, 1998

Re: Application of MURAKAMI, HIROSHI, FUJIMURA, TATSUYA, TAKAHAGI,
 YOICHI, TOYOMURA, KOJI, SHIGEHISA, TAMOTSU
 TRANSGENIC MAMMALS
 Our Ref: Q57531

Dear Sir:

The following documents and fees are submitted herewith in connection with the above application for the purpose of entering the National stage under 35 U.S.C. § 371 and in accordance with Chapter II of the Patent Cooperation Treaty:

- ☐ an executed Declaration and Power of Attorney.
- ☒ an English translation of the International Application.
- ☒ 6 sheet(s) of formal drawings.
- ☐ an English translation of Article 19 claim amendments.
- ☐ an English translation of Article 34 amendments (annexes to the IPER).
- ☐ an executed Assignment and PTO 1595 form.
- ☐ a Form PTO-1449 listing the ISR references, and a complete copy of each reference.
- ☒ a Preliminary Amendment

The Declaration and Power of Attorney and Assignment will be submitted at a later date.

The Government filing fee is calculated as follows:

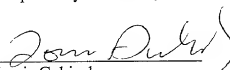
Total claims	7 - 20 =	x \$18.00 =	\$0.00
Independent claims	1 - 3 =	x \$78.00 =	\$840.00
Base Fee			\$840.00
Multiple Dependent Claim Fee			\$0.00
TOTAL FEE			\$840.00

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A check for the statutory filing fee of \$840.00 is attached. You are also directed and authorized to charge or credit any difference or overpayment to said Account. The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16, 1.17 and 1.492 which may be required during the entire pendency of the application to Deposit Account No. 19-4880. A duplicate copy of this transmittal letter is attached.

Priority is claimed from July 14, 1997 based on JP Application No. 9/205235.

Respectfully submitted,



Louis Gubinsky
Registration No. 24,835

SUGHRUE, MION, ZINN,
MACPEAK & SEAS, PLLC
2100 Pennsylvania Avenue, N.W.
Washington, D.C. 20037-3213
Telephone: (202) 293-7060
Facsimile: (202) 293-7860

Date: January 13, 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

MURAKAMI, HIROSHI, et al.

Appln. No.: Not Yet Assigned

Group Art Unit: Not Yet Assigned

Filed: January 13, 2000

Examiner: Not Yet Assigned

For: TRANSGENIC MAMMALS

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination, kindly amend the above-identified application as follows:

IN THE SPECIFICATION:

Page 1, line 1, delete "SPECIFICATION";

Page 1, after the title insert --This application is the national stage entry of

PCT/JP98/02927 filed June 30, 1998, claiming benefit of Japanese application 9/205235 filed
July 14, 1997.--

IN THE CLAIMS:

Claim 3, line 1, delete "or 2".

Claim 4, line 1, change "claims 1 to 3" to --claim 1--.

Claim 6, line 1, change "claims 1 to 5" to --claim 1--.

REMARKS

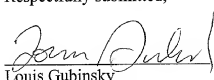
Entry and consideration of this Amendment is respectfully requested.

PRELIMINARY AMENDMENT
PCT Application JP98/02927

SUGHRUE, MION, ZINN,
MACPEAK & SEAS, PLLC
2100 Pennsylvania Avenue, N.W.
Washington, D.C. 20037-3213
Telephone: (202) 293-7060
Facsimile: (202) 293-7860

Date: January 13, 2000

Respectfully submitted,



Louis Gubinsky
Registration No. 24,835

SPECIFICATION

TRANSGENIC MAMMALS

5 TECHNICAL FIELD

This invention provides transgenic mammals. Particularly, the invention provides the nonhuman transgenic mammals carrying the human complement-inhibitor (hDAF/CD55) gene. More particularly, the invention provides domestic and laboratory animals carrying the hDAF gene.

10 BACKGROUND OF THE INVENTION

Recently, studies on animal-to-man organ transplantation (xenotransplantation) have been carried out mainly in European countries and the United States. Because of close relation to human beings, apes may be desirable donors, but the use of their organs may be infeasible because of the shortage of these animals and their high intelligence. However, domestic animals, particularly pigs, have advantages of their organ sizes and shapes similar to those of man, easy supply due to mass rearing and established basic technology. Consequently, organ transplantation from the pig to man has
15 mainly been studied.

If a porcine organ is transplanted to man, it will immediately (within minutes) and severely be rejected (hyperacute rejection), resulting in loss of its functions.

These phenomena are thought to be caused by a series of reactions: (1)

Human blood contains endogenous antibodies against porcine cells (termed natural antibodies). If a porcine organ is transplanted to man, such antibodies recognize the porcine organ and form antigen-antibody complexes. (2) The antigen-antibody complexes activate complement in human serum and trigger the complement cascade reaction. The attachment of C1 to the antigen-antibody complexes triggers reactions of C4 and C2, resulting in formation of C3 convertase, which activates C3 and cleaves it to C3b and C3a. The attachment of C3b to the cell surface of the porcine organ results in formation of C5 convertase, which activates C5 and cleaves it to C5b and C5a. The attachment of C5b to the cell surface results in sequential attachments of C6, C7, C8 and C9. (3) In consequence of the complement cascade reaction, the membrane attack complex (MAC) is formed (termed the classical complement pathway). MAC attaches the transplanted organ and causes thrombosis. (4) The alternative complement pathway is known to cause also the same cascade reaction as described above after the C3 step and finally to form MAC.

Miyagawa, S. *et al.* (Transplantation, Vol. 46(6), 825-830, 1988) reported the following: (1) the complement cascade reaction triggered hyperacute rejection of xenografts via the classical and/or alternative pathway; (2) no hyperacute rejection occurred, if the recipients had previously been treated with CVF (cobra venom factor) to cause deprivation of C3. From such findings, it has long been desired to generate transgenic animals expressing membrane-bound DAF and/or MCP, especially those homologous to recipient species, which can inhibit the cascade reaction at the C3 step.

It has been tried to generate transgenic pigs expressing a complement

inhibitor hDAF (CD55) to decompose human C3 convertase in the porcine organs (Rosengard, A. M. *et al.*, Transplantation, Vol. 59(9), 1325-1333, 1995; G. Byrne *et al.*, Transplantation Proceedings, Vol. 28(2), 759, 1996).

However, it has never been explained whether these transgenic pigs completely suppresses hyperacute rejection. Therefore, questions like the following should be answered: 1) Do these transgenic pigs express sufficient amounts of hDAF in target organs? 2) Is it necessary to co-express some other complement inhibitors? 3) Isn't it necessary to express sugar- transferase gene in order to reduce the antigen (sugar-chain antigen), which is expressed on the porcine cells and to which human natural antibodies bind? 4) Isn't it necessary to co-express the above-described gene and other genes encoding the thrombosis-preventing protein and the like? Thus, many problems are left unsolved to overcome the hyperacute rejection.

To solve these problems, it is urgent to generate pigs and/or other small-sized laboratory animals that can be handled more easily than pigs and to examine these animals from various viewpoints. Particularly, in order to carry out studies in this field and/or to develop clinical application, it is valuable to generate transgenic pigs and/or small-sized easy-to-handle laboratory animals, of which tissues and organs express hDAF of at least the same amounts as or larger amounts than those expressed in man.

Therefore, it has been tried to generate transgenic pigs expressing the human complement inhibitors as described above. Expression was examined by such methods as the following; (1) *in vitro* immunohistological examination, (2) *ex vivo* examination by allowing the transgenic pig tissues to contact directly

with human blood, or (3) *in vivo* examination by transplanting the transgenic pig tissues to primates. It was confirmed that the tissues from the transgenic pigs survived and functioned longer than those from nontransgenic pigs in *ex vivo* and *in vitro* examinations.

5 However, it was not necessarily explained whether the amounts of the human complement inhibitors expressed in the transgenic pig tissues were at least equivalent to or larger than those expressed in man.

To generate transgenic pigs expressing the human complement inhibitors, the following have been reported as the promoter genes of transgenes: (1) the
 10 promoter genes from nonporcine sources (G. A. Langford *et al.*, Transplant. Proc., 26, 1400, 1994; W. L. Fodor *et al.*, Proc. Natl. Acad. Sci. USA., 91, 11153-11157, 1994; G. W. Byrne *et al.*, Transplantation, 63, 149-155, 1997) and/or (2) the promoter genes relating to molecules distributed throughout the whole bodies of animals (e.g., beta-actin, H2K^b).

15 Transgenic mice expressing hDAF have also been generated (N. Cary *et al.*, Transplant. Proc. Vol. 25(1), 400-401, 1993; D. Kagan *et al.*, Transplant. Proc. Vol. 26(3), 1242, 1994). The loci and amounts of hDAF expressed in these transgenic mice, however, varied from report to report. Strictly speaking, no transgenic mouse expressing the human complement inhibitor in the due organ
 20 to develop it (particularly, vascular endothelial cells) in an amount larger than that expressed in human organ has ever been generated.

To solve the above problems, the present inventors studied to generate transgenic animals, particularly those other than man, expressing complement inhibitor(s) in the due organs, tissues and cells, particularly the vascular

endothelial cells, in which the complement inhibitors should essentially be expressed. The inventors succeeded in generating transgenic animals fulfilling the purposes with the promoter gene of the porcine complement inhibitor (pMCP) previously invented by the inventors (see Japanese Patent Application No. 142961/1997), by introducing the transgene designed to express the complement inhibitor(s) in the due organs, tissues and cells, particularly in the vascular endothelial cells, in which the complement inhibitors should essentially be expressed, into animals' fertilized eggs, by implanting the eggs in the uteri of recipient animals and by obtaining their youngs.

The examples described below show that the transgenic mice of this invention expressed hDAF in various organs, tissues, endothelial cells, erythrocytes, and central and peripheral nerves in amounts larger than those expressed in human cells. Furthermore, the expression of hDAF was confirmed in their erythrocytes and nerves of the transgenic pigs of the invention.

This invention was accomplished on the basis of such findings. The purpose of the invention was to provide transgenic animals useful in the medical and pharmacological fields.

DISCLOSURE OF THE INVENTION

This invention is related to nonhuman mammals carrying the human complement inhibitor (DAF/CD55) gene and expressing the inhibitor in their organs and tissues. Furthermore, the invention is related to transgenic mammals expressing the human complement inhibitor (DAF/CD55) in their vascular endothelial cells, particularly in those of all the organs and tissues.

It is favorable that the transgenic mammals of the invention are carrying the promoter gene of the porcine complement inhibitor (pMCP) at an upstream locus of the human complement-inhibitor (DAF/CD55) gene.

The transgenic mammals of this invention are useful as domestic and laboratory animals.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the structure of the transgene comprising pMCP promoter (5.4 kb) and hDAFcDNA.

Figure 2 illustrates the structure of the transgene comprising pMCP promoter (0.9 kb) and hDAFcDNA.

Figure 3 illustrates the structure of the transgene comprising hDAF promoter and hDAFcDNA used for comparison.

Figure 4 shows the PCR profiles obtained by examining the transgenic and nontransgenic mammals with hDAFcDNA-specific primers.

Lanes (1) and (3) of Fig. 4 show the PCR profiles of the hDAFcDNA-positive pig and mouse, respectively. Lanes (2) and (4) show those of the hDAFcDNA-negative littermate pig and mouse, respectively.

Figure 5 shows expression of mRNA of hDAF in various organs of a TgF1 mouse, a transgenic mouse generated for comparison and a normal mouse (nontransgenic mouse).

Expression of mRNA in various organs of the TgF1 mouse is shown in Fig. 5(A); that of the transgenic mouse for comparison (generated by introducing transgene (3) comprising hDAF promoter and hDAFcDNA) (see Fig. 3) is shown

in Fig. 5(B) ; that of the nontransgenic mouse is shown in Fig. 5(C) and that of human lymphocyte (K562) at the right end of Fig. 5(C). B, H, K, Li, Lu, S and T in each figure stand for the brain, heart, kidney, liver, lung, spleen and testis, respectively.

Figure 6 shows FACS-analysis profiles obtained by treating erythrocytes from a transgenic pig and its nontransgenic littermate pig with anti-hDAF monoclonal antibodies. Figure 6(A) shows that the erythrocytes from the transgenic pig expressed hDAF, whereas Fig. 6(B) shows that those from a nontransgenic littermate pig did not.

Figure 7 shows hemolysis profiles obtained by treating erythrocytes from the transgenic (●) and normal (■) animals with human serum. Figures (a) and (b) show the hemolysis profiles of the mouse and porcine erythrocytes, respectively. The horizontal and the vertical axes of the figure represent the complement concentration in human serum and the degree of hemolysis, respectively.

THE BEST MODE FOR APPLYING THE INVENTION

As described above, the present invention provides nonhuman transgenic mammals carrying the human complement inhibitor (referred to as hDAF in the following) and expressing the inhibitor in their organs and tissues, particularly in the vascular endothelial cells. As far as it is other than man, the species of mammals of this invention is not restricted. Examples of mammals are the mouse, rat, hamster, pig, cattle, horse, sheep, rabbit, dog, cat and so on.

Transgenic mammals of the invention can be generated by the following

methods:

First, transgene is prepared by binding promoter gene with hDAFcDNA. A part of an appropriate vector (e.g., pGL-3 basic vector, pBluescript and the like) is clipped out with a restriction enzyme(s), and the ends of the digested vector are truncated.

Base sequence encoding hDAF is clipped out from hDAFcDNA (see Medof, M. E. *et al.*, Proc. Natl. Acad. Sci. USA., 84, 2007, 1987 for example) at an upstream locus of the initiation codon and at a downstream locus of the termination codon with a restriction enzyme(s), truncated and conventionally inserted into the above-described vector. An appropriate promoter gene is also inserted at an upstream locus of the hDAFcDNA-introduced locus.

Any promoter can be used, as far as it can induce expression of hDAF in the mammals' bodies. A promoter gene of endothelin is an example. The inventors found that a promoter gene of porcine complement inhibitor (pMCP) worked more efficiently. The base sequence of the promoter gene of pMCP is defined as Sequence No. 1 (see Japanese Patent Application No. 142961/1997).

From the vector thus prepared (circular gene), transgene is prepared by digesting the region including the promoter and hDAF genes with an appropriate restriction enzyme(s).

Methods to carry out the above-described processes are commonly known by those skilled in the art. The processes can conventionally be performed.

Transgenic mammals can be generated conventionally by introducing by microinjecting the above-described transgenes into mammals' fertilized eggs (those at the pronucleus phase), implanting the eggs in the oviducts of female

mammals (recipient mammals) after a few additional incubation or directly in their uteri synchronized to the pseudopregnancy, and obtaining the youngs. If the pronuclei are hard to be recognized because of the presence of many fatty granules in the eggs, they may conventionally be centrifuged.

- 5 To find whether the generated youngs are transgenic, below-described dot-blotting, PCR, immunohistological, complement-inhibition analyses and the like can be used.

- The transgenic mammals thus generated can be propagated by conventionally mating and obtaining the youngs, or transferring nuclei (nucleus 10 transfer) of the transgenic mammal's somatic cells, which have been initialized or not, into fertilized eggs of which nuclei have previously been enucleated, implanting the eggs in the oviducts or uteri of the recipient mammals, and obtaining the clone youngs.

- As shown in the below-described examples, it was confirmed that the 15 transgenic mammals of this invention were carrying hDAF gene, expressing hDAF in the endothelial cells of all the organs and being resistant to the human complement.

INDUSTRIAL APPLICABILITY

- 20 The present invention is useful in the medical and pharmacological fields, exerting the following effects:

(1) If such organs as the heart, liver and kidney of the transgenic mammals of this invention are contacted with human blood or transplanted in primates, it can be confirmed that hDAF effectively prevents hyperacute rejection caused

by xenotransplantation.

(2) If the xenotransplantation model is prepared by contacting such organs as the heart, liver and kidney of the transgenic mammals of this invention with human blood or transplanting the organs in primates, the model will help

develop not only remedies, devices and the like to prevent hyperacute rejection after xenotransplantation but also those to prevent acute or chronic rejection after the hyperacute rejection.

(3) This invention makes it feasible to study hyperacute rejection-related problems hard to be solved only by expression of the complement inhibitors themselves. Namely, the invention may answer the questions whether it is necessary to introduce sugar transferases to reduce expression of sugar-chain antigens to which human natural antibodies bind, and/or to introduce factors to maintain homeostasis of the vascular endothelial cells (e.g., thrombomodulin, etc.).

(4) If the transgenic mammals of this invention are mated with those expressing some other complement inhibitor (human MCP or human CD59), synergic effects of the inhibitors can be examined.

(5) If the organs (e.g., the heart, lung, liver, kidney, pancreas, etc.), their adjunctive tissues (e.g., the coronary artery, endocranium, etc.) or cells (e.g., Langerhans islets producing insulin, nigrostriatal cells producing dopamine, etc.) from the transgenic mammals of this invention are transplanted to human patients whose organs have been damaged and their functions lost, they will supplement or substitute the functions of the patient organs.

(6) If the cells from the organs of the transgenic mammals of this invention

(e.g., cells from the liver, kidney and the like, Langerhans islets producing insulin, nigrostriatal cells producing dopamine, etc.) are cultured, put in an appropriate device, and connected with human patients *ex vivo*, it will supplement or substitute the functions of the damaged organs of the patients.

5

EXAMPLES

The present invention will specifically be explained in detail with actual examples, but the scope of the invention is not restricted to these samples.

10 Example 1

① Construction of transgene

The transgene comprising pMCP's promoter gene and hDAFcDNA is prepared as follows:

From pGL-3 basic vector (Promega), *luc* gene was clipped out at the *NcoI* and *XbaI* sites. Both the ends of the digested vector were truncated with T4 DNA polymerase. Next, hDAFcDNA containing the first intron was clipped out at an *AscI* site of the upstream locus of initiation codon ATG and at an *AclI* site of the downstream locus of termination codon TAG, truncated with the T4 DNA polymerase and inserted into the above-described truncated vector. Similarly, an approximately 5.4-kb region corresponding to the promoter gene was clipped out at the *EcoRI* and *FspI* sites from the porcine phage genomic library containing pMCP gene (Japanese Patent Application No. 142961/1997), and inserted into the *EcoRI* and *EcoRV* sites of the pBluescript vector.

(1) An approximately 5.4-kb promoter region inserted in the pBluescript

vector was clipped out at the *Bst*III and *Eco*RI sites (the sequence from the second to the 5,392nd bases of Sequence No. 1), truncated with T4 DNA polymerase (the sequence from the second to the 5,397th bases of Sequence No. 1), and then inserted into an *Sma*I site at an upstream locus of the above-described hDAFcDNA-inserted vector. The region containing the promoter gene and hDAFcDNA was clipped out at the *Not*I and *Eco*47III sites and used as transgene (1) (see Fig. 1).

(2) A 1.7-kb promoter region was clipped out at the *Bst*III and *Bss*H2 sites of upstream loci of the ATG initiation codon of pMCP, truncated with T4 DNA polymerase, and then inserted into the *Sma*I site of the above-described hDAFcDNA-containing vector. The vector was clipped out at the pBluescript's *Bst*XI and *Spe*I sites located at further upstream loci of the promoter and linearized. The linearized sequence was digested with a Deletion Kit for Kilo-Sequence (Takara) to obtain a deletion mutant possessing the 0.9-kb promoter gene (the sequence from the 4,498th to the 5,397th bases of Sequence No. 1). The region containing the above-described promoter gene and hDAFcDNA was clipped out at the *Not*I and *Eco*47III sites and used as transgene (2) (see Fig. 2).

(3) Transgene (3) comprising hDAF promoter gene and hDAFcDNA was prepared as follows: hDAF promoter gene was prepared by clipping out an approximately 3.8-kb region corresponding to the promoter at the *Hind*III and *Asc*I sites, truncated and inserted to an *Sma*I site at an upstream locus of the hDAFcDNA-inserted vector. A region containing the above-described promoter gene and hDAFcDNA was clipped out at the *Not*I and *Eco*47III sites and used

as transgene (3) (see Fig. 3).

Each transgene was dissolved in phosphate-buffered saline (PBS) at 5 μ g/ml before used.

5 ② Generation of the transgenic mammals (mice)

The transgenes were introduced into mouse fertilized eggs and the transgenic mice were generated as follows.

CBA or C3H male and C57BL/6 female mice were mated to obtain baby mice, of which female mice (donor mice) were used to supply fertilized eggs.

- 10 The donor mice were mated with ICR male mice after inducing ovulation (by administration of PMSG and hCG). The fertilized eggs (at the pronucleus phase) were collected. The above-described transgene (1) or (3) was introduced by microinjection into the pronuclei until their swelling was confirmed. The transgene-injected pronucleus-phase eggs were implanted in the uteri of the
- 15 recipient mice immediately after transduction or in their oviducts after additional incubation for 3 days, and then baby mice were obtained. The recipient mice were made pseudopregnancy by mating them with vasoligated male mice.

20 ③ Generation of transgenic mammals (pigs)

The transgenes were introduced into porcine fertilized eggs and transgenic pigs were generated as follows.

Fertilized eggs were collected from hybrid female pigs (donor pigs) of Landrace, Large White and Duroc. After inducing ovulation of the donor pigs (by

administration of either PMSG or FSH, and hCG) and artificial fertilization with semen of male Duroc pig, the fertilized eggs (those at the pronucleus phase) were collected. After centrifugation (for 8 min at 12,000 x g) of the pronucleus-phase eggs, transgene (2) was introduced into the pronuclei until swelling was confirmed. The transgene-injected eggs were immediately implanted in the oviducts of the recipient pigs, and then piglets were obtained. The recipient pigs were either pigs whose sexual cycle had been synchronized to those of the donor pigs by the above-described ovulation treatment or those from which the fertilized eggs had been collected.

④ Identification of the transgenic mammals

Genomic DNA was extracted from the tails of the youngs obtained from the recipient mammals and subjected to identification and selection of the transgenic mammals by the following two methods:

- (1) The dot-blotting method: Genomic DNA (10 μ g) from the youngs was placed on a piece of membrane and hybridized with gene comprising a part of biotin-labeled hDAFcDNA. The transgenic mammals were identified by detecting the introduced transgene by an alkaline phosphatase-dependent photon-generating reaction (Sumalight, Sumitomo Metal, Inc.).
- (2) PCR method: PCR was carried out (condition; denaturation for 30 sec at 94°C and annealing for 2 min and 30 sec at 68°C, 30 times) with genomic DNA from the youngs as a template, 5'-GGCCTTCCCCCAGATGTACCTAATGCC-3' of hDAFcDNA as a sense primer and 5'-TCCATAATGGTCACGTTCCCCTTG-3' as an antisense primer. The transgenic mammals were identified by detecting the

introduced transgene. The results, shown in Fig. 4, confirmed that some of the youngs obtained from the recipient mammals carried hDAFcDNA in their genome. Lanes 1 and 3 of Fig. 4 show the results with the hDAFcDNA-carrying pig and mouse, respectively. Lanes 2 and 4 of Fig. 4 those of hDAFcDNA-not-carrying littermate pig and mouse, respectively.

⑤ Propagation of the transgenic mammals (mice)

The mice confirmed to be transgenic were mated with ICR mice, and then baby mice carrying the transgene were generated (termed TgF1 mice).

⑥ Confirmation of expression of the transgene (transcription of mRNA) in the transgenic mammals (mice)

By the conventional RT-PCR method, mRNA from various organs of the TgF1 mice was examined for transcription of hDAFcDNA. For comparison, mRNA from those of the transgenic mice generated with transgene (3) comprising hDAF promoter gene and hDAFcDNA and mRNA from those of normal mice (nontransgenic mice) were similarly examined for transcription of hDAFcDNA. The results are shown in Fig. 5. B, H, K, Li, Lu, S and T in Fig. 5 stand for the brain, heart, kidney, liver, lung, spleen and testis, respectively.

With the transgenic mice generated by introducing transgene (1) comprising pMCP promoter gene and hDAFcDNA (see Fig. 1), strong signals indicating transcription of mRNA of hDAF were confirmed in all the organs examined (the brain, heart, kidney, liver, lung, spleen and testis) (Fig. 5A).

With the transgenic mice obtained by introducing transgene (3) comprising

hDAF promoter gene and hDAFcDNA (see Fig. 3), a signal of mRNA of hDAF was observed only in the testis, whereas no or faint signal in other organs (Fig. 5B).

With the nontransgenic mice, no transcription of mRNA of hDAF was observed in any organ (Fig. 5C).

- 5 With a cell line of human lymphocyte (K562), transcription of mRNA of hDAF was confirmed (the right end of Fig. 5C).

⑦ Confirmation of expression of the transgene in the transgenic mammals
(mice) (confirmation of expression of hDAF protein by an immunohistological
 10 method)

- Frozen sections of the TgF1 mouse organs were prepared and treated with biotin-labeled anti-hDAF monoclonal antibodies and then peroxidase-labeled streptavidin. After reaction with a chromogenic substrate (diaminobenzidine; DAB), the sections were microscopically examined for the intensity and the
 15 locus of the expressed hDAF protein. The results are shown in Table 1.

- With the transgenic mice generated by introducing transgene (1) comprising pMCP promoter gene and hDAFcDNA, it was confirmed that all the organs examined were intensively expressing hDAF. The organs expressing hDAF were artial and ventricular myocardia, and endothelia of medium, small
 20 and capillary blood vessels of the heart, glomerulus, uriniferous tubule, and endothelia of medium, small and capillary blood vessels of the kidney, hepatocytes, epithelia of bile ducts, and endothelia of medium, small and capillary blood vessels of the liver, alveolar wall, bronchioles epithelium, and endothelia of medium, small and capillary blood vessels of the lung, epithelia of

intestinal mucosa, and endothelia of medium, small and capillary blood vessels of the intestines, exocrine glands, Langerhans islets, epithelia and endothelia of medium, small and capillary blood-vessels of the pancreas, white and red pulp, trabeculae of the spleen, and endothelia of medium, small and capillary blood vessels of the spleen, cerebral and cerebellar cortex and medulla, and endothelia of medium, small and capillary blood vessels of the brain, seminiferous epithelia, interstitial cells, sperms, and endothelia of medium, small and capillary blood vessels of the testis and peripheral nerves.

With the transgenic mice generated by introducing transgene (3) comprising hDAF promoter gene and hDAFcDNA, the expression of hDAF was confirmed only in the testis, but not in the endothelial cells of the testis.

Table 1

Organ		Promoter gene used to generate transgenic mouse		Normal mouse
		pMCP	hDAF	
Heart	Artial myocardium	++	—	—
	Ventricular myocardium	+	—	—
	Endothelia of medium, small and capillary vessels	++	—	—
Kidney	Glomerulus	++	—	—
	Uriniferous tubule	—	—	—
	Endothelia of medium, small and capillary vessels	++	—	—
Liver	Hepatocytes	±	—	—
	Epithelia of bile duct	++	—	—
	Endothelia of medium, small and capillary vessels	++	—	—
Lung	Alveolar walls	++	—	—
	Bronchioles epithelium	++	—	—
	Endothelia of medium, small and capillary vessels	++	—	—
Intestines	Epithelia of intestinal mucosa	+	—	—
	Endothelia of medium, small and capillary vessels	++	—	—
Pancreas	Exocrine glands	+	—	—
	Langerhans islet	+	—	—
	Epithelia of pancreatic ducts	+	—	—
	Endothelia of medium, small and capillary vessels	++	—	—
Spleen	White pulp	±	—	—
	Red pulp	±	—	—
	Trabeculare lienis	+	—	—
	Endothelia of medium, small and capillary vessels	++	—	—
Brain	Cerebral cortex	++	—	—
	Cerebral medulla	++	—	—
	Cerebellar cortex	+	—	—
	Cerebellar medulla	++	—	—
	Endothelia of medium, small and capillary vessels	++	—	—
Testis	Seminiferous epithelia	++	±	—
	Interstitial cells	+	±	—
	Sperms	++	++	—
	Endothelia of medium, small and capillary vessels	++	—	—
	Peripheral nerve	+++	—	—

⑧ Confirmation of expression of the transgene in the transgenic mammals (pigs) (confirmation of expression of hDAF protein by an immunohistological method)

Expression of hDAF protein was observed in the pigs which had been

- 5 identified to be transgenic by the PCR method as described in ④.

Frozen sections were prepared from the tails of the pigs and treated with biotin-labeled anti-hDAF monoclonal antibodies and then peroxidase-labeled streptavidin as described in ⑦. After reaction with the chromogenic substrate (diaminobenzidine; DAB), they were microscopically examined for the intensity and the locus of the expressed hDAF protein.

- 10 Expression of hDAF was confirmed in the medium, small and capillary blood vessels of the transgenic pigs generated by introducing transgene (2) comprising the pMCP promoter gene and hDAFcDNA. Besides, expression of hDAF was confirmed also in such organs as the peripheral nerves, skeletal
15 muscle, and stratified squamous epithelia of the skin.

⑨ Confirmation of expression of the transgene in the transgenic mammal (pigs) (confirmation of hDAF-protein expressing by FACS analysis)

- 20 To examine for hDAF-protein expression, the organs of the transgenic pigs which had been identified to be transgenic by the PCR method as described in ④ and by the immunohistological method as described in ⑦ were subjected to FACS analysis (a fluorescence-activated cell sorter, Becton Dickinson's FACScan) with anti-hDAF monoclonal antibodies.

An erythrocyte fraction was prepared from blood of the transgenic pig,

treated with the biotin-labeled monoclonal antibodies and then Phycoprobe PE Streptavidin (Biomeda), and subjected to FACS analysis. The results are shown in Fig. 6 (A). Similar analysis as described above was carried out with a nontransgenic littermate pig. The results are shown in Fig. 6 (B). The horizontal and vertical axes represent the intensity of fluorescence indicating the amount of hDAF expressed and the cell number, respectively.

As shown in Fig. 6, it was confirmed that the erythrocytes from the transgenic pig identified by PCR and the immunohistological methods expressed huge amounts of hDAF, but that those from the nontransgenic pig did not.

Figure 6 shows also that the transgenic pigs of this example simultaneously possessed erythrocytes expressing hDAF and those not expressing hDAF (referred to as mosaic). It has already been shown that the first generation of the transgenic animals (founder) generated by the microinjection method sometimes become mosaic, and that such mosaic may disappear by such conventional methods as mating and breeding.

The results shown in ⑧ and ⑨ confirmed that the transgenic pigs generated by introducing the transgene comprising pMCP promoter and hDAFcDNA expressed hDAF from hDAFcDNA in various organs and tissues including endothelial cells.

⑩ Confirmation of expression of the transgene in the transgenic mammals (confirmation of the function of hDAF protein)

It was confirmed that the hDAF protein expressed on the transgenic

mammals' cells had the essential function of hDAF protein, *i.e.*, suppression of the complement cascade reaction. Confirmation was accomplished by determining hemolysis occurring after treating the transgenic mammal's erythrocytes with human serum. The erythrocytes were subjected to such analyses, since the complement cascade reaction could be identified by observing hemolysis (1) easily due to formation of membrane attack complex, and (2) clearly due to more fragile membrane structure of erythrocytes than other cells (*e.g.*, leukocytes, endothelial cells and the like).

The erythrocyte fractions were prepared from blood specimens taken from the transgenic and nontransgenic mouse tails and those taken from the transgenic and nontransgenic pig ear veins. After diluting the fractions with PBS, a 30- μ l portion of each fraction was placed in a well of 96-well microplates (1×10^7 cells/well), to which a 70- μ l portion of complement concentration-adjusted human serum (which had been prepared by blending human normal serum [HNS] and previously inactivated serum (by heating for 30 min at 56°C) [HIS]) was added dropwise and then allowed to react (for 1.5 h at 37°C). Optical density of the supernatant of each well was read at 405 nm with a microplate reader (Bio Rad), and the per cent hemolysis caused by the complement cascade reaction was calculated.

The results are shown in Fig. 7, in which figures (a) and (b) respectively show the results with the mouse and porcine erythrocytes. The horizontal and the vertical axes represent the concentration of HNS in human serum and the degree of hemolysis, respectively. Symbols ● and ■ in Fig. 7 show hemolysis of the erythrocytes from the transgenic and the nontransgenic animals,

respectively.

Such hemolysis occurs (1) since co-existence of animal erythrocytes and human serum immediately triggers the classical complement pathway due to the presence of the natural antibodies and complement in human serum, and (2) since animal erythrocytes (excluding the transgenic mammals of this invention) cannot inhibit human complement cascade reaction due to the species-specificity of the complement inhibitor.

As shown in Fig. 7, the erythrocytes from nontransgenic animals underwent hemolysis irrespective of the complement concentration in human serum, whereas those from the transgenic mammals inhibited hemolysis. These findings confirmed that the erythrocytes expressing hDAF from the transgenic mammals were resistant to human complement. Although the erythrocyte population of the transgenic pigs of this invention was mosaic, it was resistant to the human complement.

SEQUENCE TABLE

Sequence number: 1

Length of sequence: 5,418

Type of sequence: nucleic acid

5 Number of chains: double strand

Topology: linear

Kind of sequence: Genomic DNA

Direct origin: λ FIXII porcine genome phage library

Sequence

10	GAATTCTGCG	TACACGGGGC	CCCGGTGGCT	TTACATCATC	GCTACAGCGA	50
	CATGGGATCC	GAGCCGTGTC	TACAACCTAC	ACAACAACGC	CAGATCCTTA	100
	ACCCAATGCA	TGAGGACAGG	GCTCAAACCT	GC GGCTCAT	AGATGCTAGT	150
	CAGATTCGTT	TCTGCTGAGC	CACAATGGGA	ACTCCTAATT	CTAGATCGAT	200
	CTAGAATTAG	GAGTTCCTCAT	TGTGGCTCAG	CAGAAACGAA	TCTGACTAGC	250
15	ATCTATGAGG	CGCGAGTTTG	AGCCCTGTCC	TCATGCATTG	GGTTAAGGAT	300
	CTGGCGTTGT	TGTGTAGGTT	GTAGACACGG	CTCGGATCCC	ATGTCGCTGT	350
	AGCGATGATG	TAAAGCCACC	GGGGCCCCGT	GCTACGCGAG	ATTCNTGCAG	400
	CCCGGGGGAT	CCACTAGTTC	TAGCNAGAGA	GTGAAAAATT	TAAAGAACAT	450
	TTCTCCCCTA	ATCTCCCAA	ATATGGGCAA	AGGACAGGTA	CCCGTGGGAC	500
20	TGGAAAAATA	CAGGCAAGCA	ACCCATGAGT	ACATGAAAG	ATGTCACAGG	550
	GTTCCGGCTA	ATGGAAGCCT	GAACAATGCC	TATCACATCG	TGGGTTTCTG	600
	AAGAAGTAAC	TAAAGAAAC	TAGAAATTAA	ATGGCTTTCT	TAGAATGAAA	650
	ATTCTCTATC	ACAAGGAAAA	ATGTTGTATG	TTGTTTTTCC	CATAATGGAG	700
	GTCAATGGGC	GCTATGATTA	ACAAATATCT	GATGCTGTG	ACTTTTTAAT	750
25	TGCAAGAAAT	CTGTGNAGTT	TTTTTATTAT	CTATGGGAAA	TATTGCATAT	800
	ATTAATGATA	TCACCTAACT	TGTATTATTG	AGCAATTCTG	TCCACATCTG	850
	GCCTTTCATC	TTTCATCTAA	AAAGCAGGGG	CTGGACCAAC	TGACCTTCAG	900
	TGCCATTCTT	ACTGCTAACA	TTCTAATTTT	GTTTTTATTG	CCTTTTTGTA	950
	CAAAAGCTGT	AGAGAAAGTCA	TTTTAAGTCT	GTGACATTAA	ATGTAATTTT	1000
30	CTGTCTCCAG	CATTATAATA	AGAATCAAAG	ATTTAATCTA	ATACACCGAT	1050
	GGAAATATTGT	TTATAACGTA	TTTACTGTTT	CAAGCCTTCA	AAACCAAGAG	1100
	AAAACAAAAT	GAGTACCTGT	TCCTTCTGAG	AAATGCCCTT	CTTCCTGTTC	1150
	AGAATCCCTG	TGTATAACAG	GAATGCTCTC	GAGTTAACAG	CCAAGTAAGA	1200
	GGCCCATCGG	CTGGCAGGTG	CCCACCTAGC	TAGGTGCAAG	CAGAGGTGGC	1250
35	AGTGCTCCCA	GGACCAACAG	CAGAAACATG	GCTTAACTAT	CCTGTGTTTA	1300
	GCAGTTCTCT	TACGGGTTTT	CACAACACCT	AAAAAGCGCC	CTGATGGGGT	1350
	AAAGCCTCTG	CCTTCATGCT	GCTGCCCGT	CTCTGAAAA	CAGGACGTAA	1400
	ATATACAATT	TAGGAGGTAA	GAGGGACATC	TGCCATTGTT	TTCTTTAAAC	1450
	CAGTCAGCCT	CTGTTTAATG	AATCCCAGCC	ACCTCCCTCC	ACCTACCATC	1500
40	ATTCCTAAAG	TTTGACAGAG	AGCTGCCATA	GAGCTCAAAA	CACGGWNTAC	1550

	AGACAAGCAT	NTTCTCCATC	CCTCCTCATC	TTCTCACAGG	CCGCTTGACA	1600
	ACATCTCTAG	GAGGGGGTGG	AGGCGCCACC	AGTGTTCGAG	CCCCTCGTTC	1650
	ACGCAAAAGCC	TTGACTCTGG	AGTTCTAGTC	CTCGCGGGAC	CTTAGGAAAGT	1700
	TCACGGTCAA	TACTCCGCCC	TTGGGCTCAG	ACACTAAGAG	GATCTCCGGG	1750
5	TAAGAAGATA	GACAGTAGCT	CCATGCCTGA	TTTAGGAAAA	GCTCCGCTAC	1800
	AGACAGTTGT	AATTCAATTC	TTTCAGAGAG	AAATCCTGCT	CTGTCTCGTAG	1850
	TTCTGAAAGT	CATTAAAAATC	AAAAGCTCTC	AGAAACGTCC	CAGCATTTGC	1900
	TAAGTCCACG	CTGGGGGAGG	ATGGGCGAGAG	CCGTGTTTCAG	CGCGTTTGAC	1950
	AGCAACACCC	ACTTATTTTC	TTYAGTATCC	ATAGGCATAT	ATCATGCAAC	2000
10	TGGTATAGGC	CTCTCTCTCA	GCACCTGGAG	TACAGCAAGA	AAACGCTATT	2050
	CCTGCCCCAT	GGAGCGTTGTW	MARAAAATAA	GANNNAAAAA	CGCTTTANAA	2100
	ANGGAAGCTR	CCNGMTGGGN	CMAAGTNAAA	ATTAAGTAAA	AAGAAAWCCG	2150
	TGARRAAACC	CTTCAGTNAT	ATTAAGAAAG	AAANTAGCTT	GATGAAACCC	2200
	CAGGTGTANA	AATTNNCACT	AAAACATAGS	TCCCAATTA	AAACCCCMMA	2250
15	TTCATGGAAT	TTTACTNAGT	ANGCTGNAAC	TAGGAAACCC	AACTTCTAGC	2300
	CNATAGTTTC	TCCCTTCTAA	ATNTTGTCTAT	GAGAAAAACA	YTTATTTCCA	2350
	AAGANATTTT	CCATGATGGG	GAAAGTTTTT	TTCAACTTTG	CTCAGGTATA	2400
	AACTGAANAT	ACAGCATATA	AGTAAAGATA	GTTCAGAGAG	GCACCAATA	2450
	GATACCCGTT	TTCAAAAAAA	GTGCCAACAT	GGAGCCAGAG	AAATTTCCG	2500
20	TTACATCACG	CTTTTACGGC	TTTGAATAAT	ACACGAGATG	ATCATCCCCC	2550
	MCCTTGGGTT	TCCNACTCCN	TCCCTCCTNA	ATTTTACCTC	CTTTAATTGT	2600
	CATCATGTCT	GGAGATTATA	ATCCAAGATA	CTAAGATGTT	TATNTCATAC	2650
	ATCGCCTCCA	CACAGGTGTT	CTNANAAGCT	CTTGCAAGAA	TCCAAACATT	2700
	GTGCTGGTCT	GGGTAGAAAA	GGAAATTCCA	TGGTTTGTG	AACCCAGGAA	2750
25	CTCTTCAGTA	CATCTCCGAG	GTAAACTGT	TTAAATACAA	TTAAAGTTCT	2800
	ACAGTTAAAG	GGTACCCTCC	TCCACTGTTG	GTGGGAATGT	AAACTGGTAC	2850
	AATCACTATT	AAAAACAGGA	TGGAGGTACT	TCAGAAAAATG	AAGTATAGAA	2900
	CTACCACAGG	ATCCAGCACT	CTCACTCCTG	GGCACCTATC	AGGACAAAAA	2950
	ATTCCGTCGA	AAAGATGCAT	GCACCCATAG	CTATGTTTAC	TGCACAGCA	3000
30	TTCAACAATG	CCAAGACATG	GAAACGACCT	AAATGTCCAT	CACACGCTGA	3050
	ATGCATTAAG	AAGACGTGGT	ATATACACAC	AATGGAATAC	TACTCAAATC	3100
	ATGAAAAAGA	ACAAAAAGAA	GCCATTTGCA	GCAACATGGC	ATGGCTGGAA	3150
	CTAGAGACTC	ATGCTAAATG	AAGTCAGTGA	GAAAGAGAAA	GACAAAAATCC	3200
	ACATGATATC	ACTTATATCT	GGAATCTAAT	ATACGACAGA	CATGAAACTT	3250
35	TCCACAGAAA	AGAAAAACCTN	CATGGAATCT	GGAGAACAGA	CTTGTGGTTT	3300
	CSCCAAGGGG	GGARGGGGGG	AAGACCGTGG	GAGGACTGGG	GAGCTTTGGG	3350
	GTTAATAGAT	GCAAAAACTAT	TGCCTTTNGA	ATGGATAAGC	CAATGGGATC	3400
	CTGCTGTACC	AGAACCRRGGG	AACATATNCT	AGTCACTTGG	KNTAGAACAT	3450
	GATGGAGGAT	NATNTGAGAN	AAAAAGATATN	TGTGTGTGK	AGAGAGAGAG	3500
40	AGACTGGCTC	CACCTTTGCTG	TATAGTAGAA	AACTGACAGA	ACACCGTAAA	3550

	CCATTAATA	AAAATCCAGT	AAAAATTTAA	AAATAAAAAAC	ACACATTGGT	3600
	TCCAATGTGT	TTAAAAGCAA	TAAAGTTCTA	TAATTGCAGC	AGATGCATCT	3650
	GAGGTTTACA	CGGAGAGCTT	CCATTCTCTA	CCATCCTCTC	ATTCCTTAAC	3700
	TCTAATGTGA	TACAGGTTCT	ATTCTCACCA	TTCTATGAAC	AAAAGAGCAG	3750
5	CTGATTTACA	GGTTGGATTT	TTCAAAAAAA	AAAATTTCTT	TACCAGGATC	3800
	CCAAATGTAA	CAAAGGGTCA	ATATAGAAAA	CTTAAAAAGC	ACAGCCAAAG	3850
	AGAAATATAC	ATAAGCCTTT	CAACTATTAA	TTTTGATTAA	TATCCAACGA	3900
	ATCTCTTTTT	AAGTGTATCA	ATATATTATT	CATTTTAATA	AAAGAAATTG	3950
	CAAGAGGCAC	TTGCTTTTTT	TGCTTACAAA	TACGGTTTCT	CAAATCGATT	4000
10	TTTTTTATAT	ACTGTTTGCA	TAGAATTTCA	ATCCATAAAG	CTACCTATTG	4050
	AAAATTCCTT	ATATTTCTGC	TAAACACTTA	AGGGCTTATA	TTTTCTCCAA	4100
	ATTTATACAT	CCTTGCTCAC	AGTTCTGACG	ATGTCTTTGG	GATAAACTCT	4150
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	AAAAAAGGTA	AGTTAAAAAG	TAAAGTTTGG	GGGAGGCTGC	TGGTCGCCCC	4250
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	GTCAATGTCT	TTTTTACTG	CTTAATACTG	GTCAATGTAC	TTTTCTTTCC	4350
	TTTTGCTAAT	CATACAGGCT	TACTCACAAAC	TCTACAAAAA	AATCTTACTC	4400
	ATTCCTAATG	TTCCCTTCATT	GAGAGATTGG	TTTGCGGGAA	ACGTTCTCAC	4450
	TCTCACCAAG	TCCCAACAGT	CCCAACTCTA	ACGACGGTCG	CTGCTTCCAG	4500
20	AAATACGGCA	CTTAAGGCAC	CCTCGTCCTT	ACCTTTTTCA	TGCATGTGTA	4550
	TTTCATTTTC	AATAAAACAT	TGAGTTGTTT	CAAGGCCAGA	CCATAGAGTT	4600
	GAGCCCAAC	ATGCTAGTGG	CCCAGTGTGA	TGTAATAATT	TACCTTCCCA	4650
	GGGGTCCTCT	CCGGGGGGGT	ACAGGCGAGA	CTAAGTGACT	TTAAGCTGTT	4700
	GGGAGAACAA	TGGCCAAACC	TTTCGTGATT	TTGAAATCTA	TCAGGCCACG	4750
25	AGACACTTCG	GTAGCGGACG	CTCAACCCTG	GGAAATCCCA	CTATTGTCCC	4800
	AAATTTTGCC	TGACTCGTGC	CAAAGATTGA	GCCAGGGCCC	GGGTGTCCAG	4850
	GCAGTCTGCA	GTGCCCCAGT	CCCCACCAGA	GCCCTGAAGG	GTGTCGGGCC	4900
	CCACGAAACC	GCTGCCCGGG	CTCTAGGGTT	TCTGTTTTCA	GGTCGCTGCG	4950
	CTTTATTCTC	TAATTACGCG	TTCCCGAAAG	AGACCATTGAG	GACCCGCCCA	5000
30	GTGTCCCTTA	CACCTTCCCG	TGTCGGGTGG	CGACAGCTGT	TTACGAAGAA	5050
	GAGTGCACCA	CCCTTTCCCG	CAAGCCGCGAG	CGGTTAGTTT	CGCAGAAGGA	5100
	GGAGCCAGGG	CGTCGGGGCG	CAGCTGGGAG	AGAGGCCCGG	CAGCGGGCGC	5150
	CGCGGACGAG	CAAGGGCGTC	CCTCTCTCGG	CGGGAGCCCC	GCCCCGCCCG	5200
	GCCCCACGG	CCCCGCCCTT	CGGCCCGCCC	ATTGGCTCCG	CGGGGCCCTG	5250
35	GAGTCACTCC	CTAGAGCCAC	TTCCGCCCAG	GGCGGGGCCC	AGGCCACGCC	5300
	CACTGGCCTG	ACCGCGCGGG	AGGCTCCCGG	AGACCGTGGA	TTCTTACTCC	5350
	TGCTGTGCGA	ACTCGAAGAG	GTCTCCGCTA	GGCTGGTGTC	GGGTTACCTG	5400
	CTCATCTTCC	CGAAAAATG				5418

CLAIMS

1. Transgenic mammals other than man carrying gene of a human complement inhibitor (DAF/CD55) and expressing the human complement inhibitor in their organs and tissues.
2. The transgenic mammals as claimed in claim 1, in which they express the human complement inhibitor (DAF/CD55) in their endothelial cells.
3. The transgenic mammals as claimed in claim 1 or 2, in which they express the human complement inhibitor (DAF/CD55) in their endothelial cells of organs and tissues of the whole body.
4. The transgenic mammals as claimed in claims 1 to 3, in which they carry promoter gene of the porcine complement inhibitor (pMCP) at an upstream locus of the human complement inhibitor (DAF/CD55) gene.
5. The transgenic mammals as claimed in claim 4, in which the promoter gene comprises the porcine complement inhibitor (pMCP) promoter defined by Sequence No. 1 or its parts.
6. The transgenic mammals as claimed in claims 1 to 5, in which they are domestic or laboratory animals.
7. The transgenic mammals as claimed in claim 6, in which they are transgenic pigs or transgenic mice.

ABSTRACT

This invention provides transgenic mammals other than man carrying the gene of the human complement inhibitor (DAF/CD55) . and expressing the human complement inhibitor in their organs and tissues, particularly in their endothelial cells. This invention provides nonhuman transgenic mammals useful as laboratory animals in the medical and pharmacological fields and/or sources of organs, tissues, cells and the like for medical treatment of man.

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15

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Fig.1

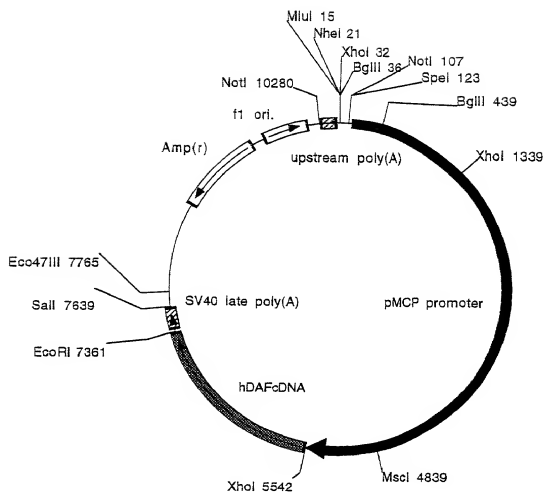


Fig.2

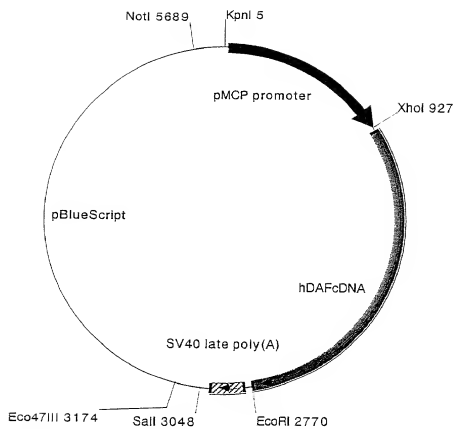


Fig.3

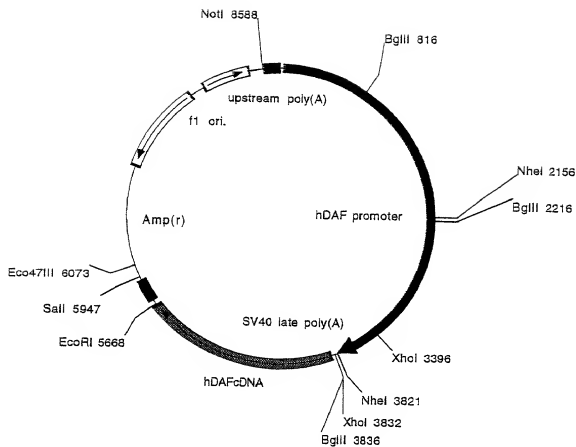


Fig.4

1 2 3 4



Fig.5

B H K LiLuS T

(A)



B H K LiLuS T

(B)



B H K LiLuS T

(C)



K562

Fig.6

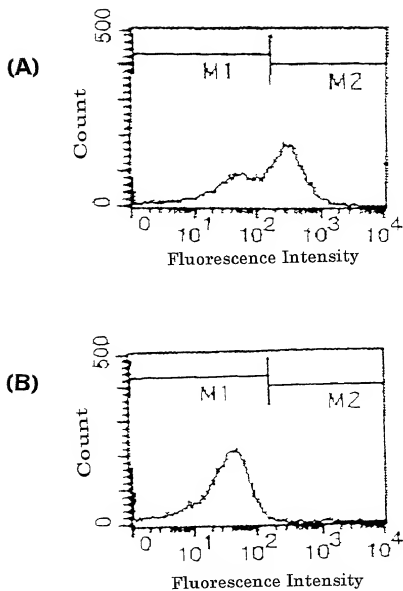
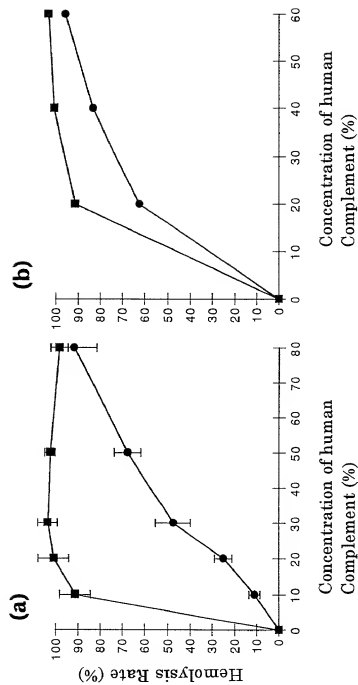


Fig. 7



Declaration and Power of Attorney for Patent Application

特許出願宣言書及び委任状

Japanese Language Declaration

日本語宣言書



下の氏名の発明者として、私は以下の通り宣明します。

As a below named inventor, I hereby declare that:

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My residence, post office address and citizenship are as
stated next to my name.

下記の名称の発明に関して請求範囲に記載され、特許出願
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と（下記の名称が複数の場合）信じています。

I believe I am the original, first and sole inventor (if only
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inventor (if plural names are listed below) of the subject
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TRANSGENIC MAMMALS

上記発明の明細書（下記の欄でx印がついていない場合は、
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- ☐ 月 日に提出され、米国出願番号または特許協定条約
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☒ was filed on June 30, 1998
as United States Application Number or PCT
International Application Number
PCT/JP98/02927
_____ (if applicable)

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Prior foreign application(s)
外国での先行出願

09/205235
(Number)
(番号)

JAPAN
(Country)
(国名)

14/7/97
(Day/Month/Year Filed)
(出願年月日)

Priority Not Claimed
優先権主張なし

☐

(Number)
(番号)

(Country)
(国名)

(Day/Month/Year Filed)
(出願年月日)

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(Number)
(番号)

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(国名)

(Day/Month/Year Filed)
(出願年月日)

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(出願日)

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(現況：特許許可済、保属中、放棄済)

(Application No.)
(出願番号)

(Filing Date)
(出願日)

(Status)(patented, pending, abandoned)
(現況：特許許可済、保属中、放棄済)

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書類送付先

Send Correspondence to:
SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC
2100 Pennsylvania Avenue, N.W. Suite 800
Washington, D.C. 20037-3213

直接電話連絡先: (名前及び電話番号)

Direct Telephone Calls to:

(202)293-7060

唯一または第一発明者名	Full name of sole or first inventor Hiroshi MURAKAMI
発明者の署名 日付	Inventor's signature Date H. Murakami Apr. 3, 2000
住所	Residence
国籍	Citizenship Japanese
私書箱	Post Office Address c/o Nippon Meat Packers, Inc., Research and Development Center, 3, Midorigahara 3-chome, Tsukuba-shi, Ibaraki Japan 300-2646 JAPAN
第二共同発明者	
第二共同発明者 日付	Full name of second joint inventor, if any Tatsuya FUJIMURA
住所	Second inventor's signature Date T. Fujimura Apr. 3, 2000
国籍	Residence
私書箱	Citizenship Japanese Post Office Address c/o Nippon Meat Packers, Inc., Research and Development Center, 3, Midorigahara 3-chome, Tsukuba-shi, Ibaraki Japan 300-2646 JAPAN

(第三以降の共同発明者についても同様に記載し、署名をすること)

(Supply similar information and signature for third and subsequent joint inventors.)

Japanese Language Declaration

日本語宣言書

第三の共同発明者の氏名 (該当する場合)		Full name of third joint inventor, if any <u>Yoichi TAKAHAGI</u>
同第三発明者の署名	日付	Third inventor's signature <u>Y. Takahagi</u> Date <u>Apr. 3, 2000</u>
住所		Residence
国籍		Citizenship
郵便の宛先		Japanese Post office address c/o Nippon Meat Packers, Inc., Research and Development Center, 3, Midorigahara 3-chome, Tsukuba-shi, Ibaraki Japan 300-2646 JAPAN
第四の共同発明者の氏名 (該当する場合)		
同第四発明者の署名	日付	Full name of fourth joint inventor, if any <u>Koji TOYOMURA</u>
住所		Fourth inventor's signature <u>K. Toyomura</u> Date <u>Apr. 3, 2000</u>
国籍		Residence
郵便の宛先		Citizenship Japanese Post office address c/o Nippon Meat Packers, Inc., Research and Development Center, 3, Midorigahara 3-chome, Tsukuba-shi, Ibaraki Japan 300-2646 JAPAN
第五の共同発明者の氏名 (該当する場合)		
同第五発明者の署名	日付	Full name of fifth joint inventor, if any <u>Tamotsu SHIGEHISA</u>
住所		Fifth inventor's signature <u>T. Shigehisa</u> Date <u>Apr. 3, 2000</u>
国籍		Residence
郵便の宛先		Citizenship Japanese Post office address c/o Nippon Meat Packers, Inc., Research and Development Center, 3, Midorigahara 3-chome, Tsukuba-shi, Ibaraki Japan 300-2646 JAPAN
第六の共同発明者の氏名 (該当する場合)		
同第六発明者の署名	日付	
住所		
国籍		
郵便の宛先		